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[COMMUNICATION]

Effect of Arginine Vasotocin (AVT) and AVT-Related Peptide on Skin Gland Secretion in *Xenopus laevis*

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ABSTRACT—Arginine vasotocin (AVT) and AVT C-terminally extended with glycine (AVT-Gly) were isolated from the neurointermediate lobes of the bullfrog, *Rana catesbeiana*, using C_{18} SEP-PAK cartridges, and Sephadex G-50 and reverse-phase HPLC columns. These peptides exhibited marked aldosterone-releasing activity when injected into *Xenopus* juveniles. AVT-Gly (0.1–5 μg) as well as AVT (0.01–5 μg) injected subcutaneously into *Xenopus* juveniles (8 g body weight) stimulated the discharge of a secretory material presumably from the granulated glands in the skin, AVT-Gly being less potent than AVT. Both peptides also induced defecation in the animals, the incidence of defecation increasing with higher dosage.

INTRODUCTION

The neurointermediate lobe of amphibians contains potent stimulators of aldosterone release from the adrenocortical tissue [1]. Recently, we have isolated two active peptides from the neurointermediate lobes of the bullfrog and identified them as arginine vasotocin (AVT) and AVT C-terminally extended with glycine (AVT-Gly), apparently derived from a pro-vastocin-neurophysin precursor [2]. Both exist in the hypophyseal tissue in almost equal quantities and exhibit equipotent activity in stimulating water-flux from the isolated urinary bladder of the Japanese toad and the release of aldosterone from the adrenals of *Xenopus* [3]. AVT-Gly has also been isolated from the neurointermidiate pituitary glands of *Rana esculenta* and termed hydrin 2, since, like AVT, it increases water uptake from hypotonic bathing solutions through the skin of frogs [4]. According to Ireland [5], AVT causes an intense skin gland secretion in *Xenopus*. In the present paper, we report that both AVT-Gly and AVT induce discharge of secretory material from the skin glands, and cause defecation in *Xenopus*.

MATERIALS AND METHODS

Preparations of AVT and AVT-Gly

AVT and AVT-Gly were purified from an acid-acetone extract of the neurointermediate lobes of the bullfrog (*Rana catesbeiana*) by elution with C_{18} SEP-PAK cartridges (Waters), a Sephadex G-50 column (Pharmacia) and reverse-phase HPLC columns (TSK ODS120T, TOSOH) as described previously [3]. The final preparations were checked for their aldosterone-releasing activity *in vivo* with *Xenopus laevis* juveniles (about 8 g b.w.), according to the previously described procedures [3]. Radioimmunoassay for aldosterone in the plasma was carried out using standard aldosterone (MERCK), [^{3}H]-aldosterone (New England Nuclear Co.) and anti-aldosterone serum (Miles-Yeda). The details have been described elsewhere [6].

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Dermal gland response

Xenopus juveniles weighing 6–8 g were injected subcutaneously with various amounts of AVT or AVT-Gly dissolved in 50 μl of saline. Each animal was kept separately in a glass container (15 cm in diameter). Dermal gland responses to the peptides were evaluated by observing the dorsal side of the skin with the naked eye for 60 min. Occurrence of defecation during the observation period was also recorded.

Histological study

After a 60 min of observation period, the animals were sacrificed by decapitation. A piece of dorsal skin (0.5×0.5 mm) was taken from each specimen, fixed in Bouin's fluid, processed routinely for light microscopy and stained with PAS-azan.

RESULTS AND DISCUSSION

Both AVT and AVT-Gly prepared for the present experiments exhibited marked aldosterone-releasing activity. Hypophysectomized *Xenopus* juveniles received a subcutaneous injection of 0.1 μg of AVT or AVT-Gly. Plasma aldosterone levels measured 20 min after the treatment were significantly higher ($P<0.05$; Duncan's new multiple range test) in AVT-treated (14.10±1.80 ng/ml, n=5) and AVT-Gly-treated (11.42±2.38 ng/ml, n=5) groups than in the saline-injected controls (1.17±0.43 ng/ml, n=5). There was no significant difference between the value for the AVT-treated group and that for the AVT-Gly-treated group. The results were consistent with the previous ones, which showed that AVT and AVT-Gly stimulated aldosterone release from the adrenals of *Xenopus* both *in vivo* and *in vitro* [3]. Thus, it was confirmed that both preparations possessed bioactivity.

Within a few minutes after subcutaneous injection of a suffficient amount of AVT or AVT-Gly, discharge of a milky-white material from the skin glands was observed. Both peptides in relatively high doses caused intense secretion, and the secretion became less conspicuous as the dose was decreased. As shown in Table 1, AVT appeared to be more potent that AVT-Gly in stimulating the discharge of the secretory material. On the other hand, the aldosterone-releasing activities of the AVT and AVT-Gly preparations did not differ under the present experimental conditions. According to our previous results [3], AVT and AVT-Gly also showed equipotent activity in stimulating aldosterone-release from the adrenals of *Xenopus* and water-flux from the isolated bladder of the Japanese toad.

In *Xenopus laevis,* two types of skin gland are known: the granulated gland and the mucous gland [7, 8]. In addition, two other types of gland have recently been described by Fujikura *et al.* [9], and designated the small granulated gland and the NP gland. NP glands are found only in the nuptial pad of the male forelimb, whereas the others are widely distributed over the body surface. In the

Table 1. Induction of skin gland secretion and defecation by AVT and AVT-Gly

amount injected (μg)	AVT			AVT-Gly		
	++	+	−	++	+	−
5	3	2	0 (4)*	4	1	0 (4)
1	5	0	0 (5)	4	1	0 (5)
0.5	3	2	0 (3)	2	2	1 (1)
0.1	3	2	0 (1)	0	3	2 (1)
0.05	0	4	1 (1)			
0.01	0	0	5 (0)			

++, more than 50% of the dorsal surface was covered with a milky-white secretory material; +, less than 50% of the dorsal surface was vovered with a milky-white secretory material; −, absence of any milky-white secretory material on the dorsal surface.
* Incidence of defecation during a 60-min observation period.

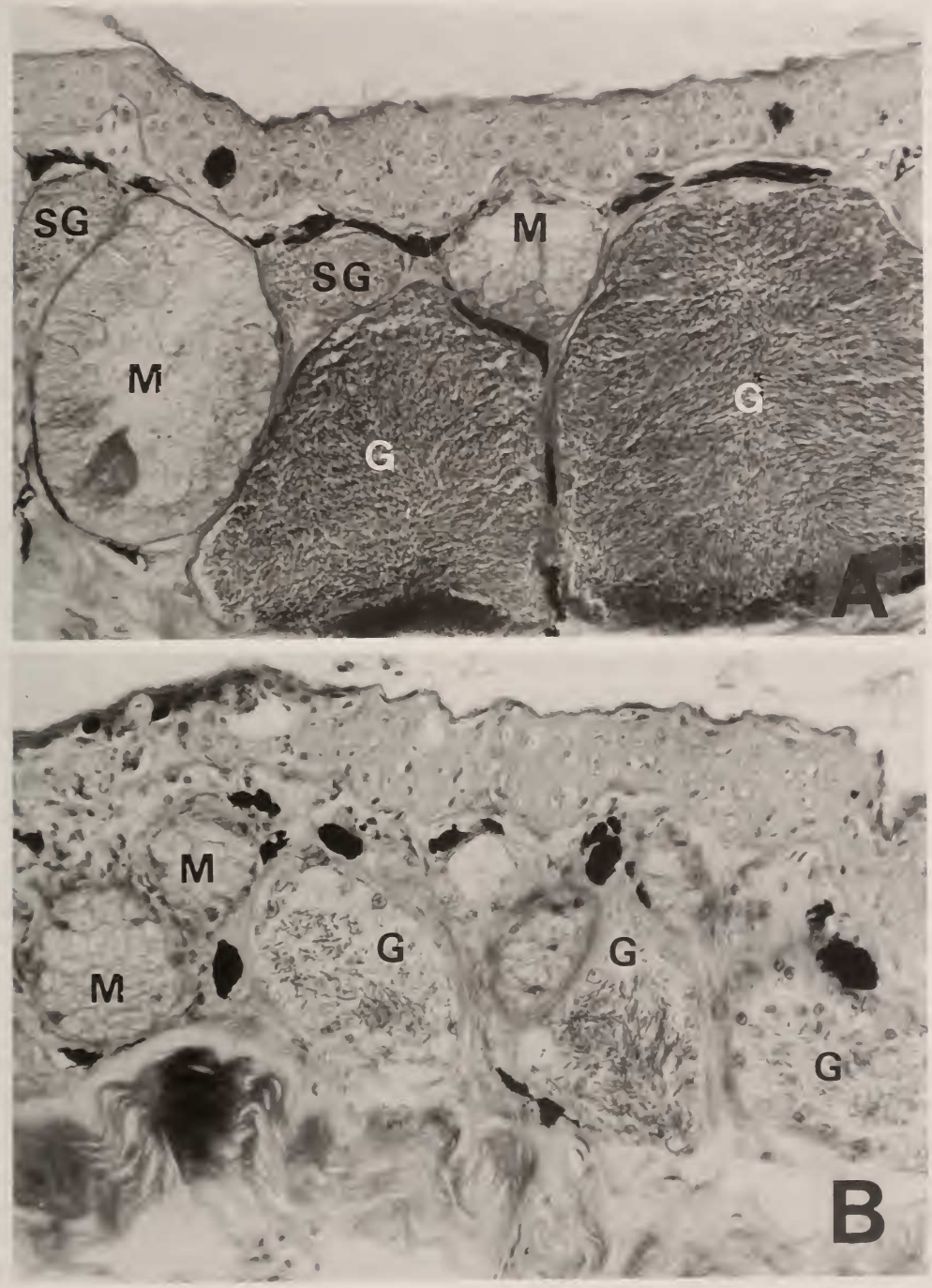

FIG. 1. Histological view of skin glands of the saline-injected (A), and AVT-Gly (5 μg)-injected (B). G, granulated gland; M, mucous gland; SG, small granulated gland. $\times 300$.

specimens which discharged a massive milky-white material in response to AVT or AVT-Gly, the granulated gland contained less azocarmine-positive material and the musculature surrounding the gland exhibited contraction. The effect of the peptide on the mucous and small granulated glands was less conspicuous. However, the musculature surrounding them seems to be contracted (Fig. 1B). The results were consistent with those obtained by Ireland using synthetic AVT [5]. In the animals which was not evoked skin gland secretion with relatively low dosage of AVT or AVT-Gly, the granulated glands were large and rich in the secretory material as in the saline-injected controls (Fig. 1A). The present results indicate that AVT-Gly is less potent than AVT in inducing contraction of the smooth muscle surrounding the granulated glands.

In parallel with the response of the skin glands to AVT and AVT-Gly, the incidence of defecation from the cloaca was increased (Table 1). Neurohypophyseal peptides are known to induce contraction of the oviducts of vertebrates other than mammals and AVT appears to be especially active [10]. It is of interest to note that in *Xenopus,* AVT-Gly as well as AVT elicits defecation presumably by inducing contraction of the intestinal musculature.

According to Rouillé *et al.* [4], the neurohypophysis of Bufonidae and Ranidae contains AVT-Gly in addition to AVT, whereas that of *Xenopus* contains AVT and AVT C-terminally extended with the Gly-Lys-Arg sequence (hydrin 1). Whether the latter peptide also stimulates aldosterone release, skin gland secretion and defecation remains to be clarified.

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